



TITLE:

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AUTHOR(S):

Hayakawa, Takao; Aoi, Takashi; Umezawa, Akihiro; Ozawa, Keiya; Sato, Yoji; Sawa, Yoshiki; Matsuyama, Akifumi; Yamanaka, Shinya; Yamato, Masayuki

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## Original article

# A study on ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of allogeneic human somatic stem cells<sup>☆</sup>



Takao Hayakawa<sup>a, \*</sup>, Takashi Aoi<sup>b, c</sup>, Akihiro Umezawa<sup>d</sup>, Keiya Ozawa<sup>e</sup>, Yoji Sato<sup>f</sup>,  
Yoshiki Sawa<sup>g</sup>, Akifumi Matsuyama<sup>h</sup>, Shinya Yamanaka<sup>i</sup>, Masayuki Yamato<sup>j</sup>

<sup>a</sup> Pharmaceutical Research and Technology Institute, Kindai University, Japan

<sup>b</sup> Department of iPS Cell Applications, Graduate School of Medicine, Kobe University, Japan

<sup>c</sup> Center for Human Resource Development for Regenerative Medicine, Kobe University Hospital, Japan

<sup>d</sup> Department of Reproductive Biology, National Research Institute for Child Health and Development, Japan

<sup>e</sup> Division of Genetic Therapeutics, The Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Japan

<sup>f</sup> Division of Cell-Based Therapeutic Products, National Institute of Health Sciences, Japan

<sup>g</sup> Division of Cardiovascular Surgery, Department of Surgery, Osaka University Graduate School of Medicine, Japan

<sup>h</sup> R&D Division of Regenerative Medicine, Foundation for Biomedical Research and Innovation, Japan

<sup>i</sup> Center for iPS Cell Research and Application, Kyoto University, Japan

<sup>j</sup> Advanced Biomedical Science Center, Tokyo Women's Medical University, Japan

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## ABSTRACT

As a series of endeavors to establish suitable measures for the sound development of regenerative medicine using human stem cell-based products, we studied scientific principles, concepts, and basic technical elements to ensure the quality and safety of therapeutic products derived from allogeneic human somatic stem cells, taking into consideration scientific and technological advances, ethics, regulatory rationale, and international trends in human stem cell-derived products. This led to the development of the Japanese official Notification No. 0907-3, "Guideline on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Allogeneic Human Somatic Stem Cells," issued by Pharmaceuticals and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan, on September 7, 2012. The present paper describes the background information and development of our study and the resulting guidance. For products derived from allogeneic somatic stem cells, major points to consider include 1) history, the source, and derivation of starting cells; 2) donor screening/testing and donor eligibility, especially in relation to the presence of adventitious agents, potential occurrence of donor-derived diseases, and immunocompatibility; 3) clinical records of a donor; 4) multipotency and self-replication ability of allogeneic human somatic stem cells; 5) cell banking; 6) potential presence of viruses in the final product; 7) extensive characterization of the cells at critical stage(s) of manufacture; 8) robustness of the manufacturing process; 9) quality consistency of the products such as the final products and critical intermediate(s) if any; and 10) robust application and function of the final products in a cell environment different from where the original cells were localized and were performing their natural endogenous function. The ultimate goal of this guidance is to provide suitable medical opportunities as soon as possible to the patients with severe diseases that are difficult to treat with conventional modalities.

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<sup>☆</sup> Recently, this type of product has been defined as a distinct product from both conventional pharmaceuticals and medical devices according to the revised Pharmaceutical Affairs Law -renamed the Pharmaceuticals and Medical Devices, and Other Therapeutic Products Act. (Akinori Hara, Daisaku Sato, and Yasuyuki Sahara: New Governmental Regulatory System for Stem Cell–Based Therapies in Japan. *Therapeutic Innovation & Regulatory Science*. 2014; **48**(6): 681–688.)

\* Corresponding author.

E-mail addresses: [takao-hayakawa@mtg.biglobe.ne.jp](mailto:takao-hayakawa@mtg.biglobe.ne.jp), [hayakawatakao@gmail.com](mailto:hayakawatakao@gmail.com) (T. Hayakawa).

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## 1. Background (chronology and focus of the research)

The details of the present study were described in a previous paper<sup>1)</sup>. The present paper summarizes points that are closely related to those presented in the earlier paper.

Regenerative medicine using cell-based products that are derived from the processing of human cells and tissues is keenly anticipated in Japan because of difficulties with securing human organs and tissues in our country. With technology breakthroughs and research advances, people are increasingly hopeful that medical technology using novel cell-based products will develop into new therapies.

In Japan, translational research to regenerative medicine is advancing rapidly. In particular, considerable work has been done to develop products that make use of human stem cells, i.e., somatic stem cells such as mesenchymal stem cells, embryonic stem (ES) cells, and induced pluripotent stem (iPS) cells. Thus, there is an urgent need to prepare relevant guidelines on the evaluation of products expected in the near future. Identifying at an early stage of development the technical, medical, and ethical conditions necessary for the utilization of various types of stem cells at an early stage of development is vital for their rapid application to the treatment of patients.

In the fiscal year 2008, the Japanese Ministry of Health, Labour and Welfare convened a panel of experts: the “Study Group on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Human Stem Cells.” The panel was established as a scientific research project of the Japanese Ministry of Health, Labour and Welfare and has been chaired by Dr. Takao Hayakawa since its conception.

The objective of the study group is to promote the sound development of products derived from human stem cells by investigating scientific and technological advances, ethics, the regulatory rationale, and international trends regarding human-stem-cell-derived products and to establish and implement appropriate safety evaluation criteria.

As a result of analyses conducted up to 2009, in accordance with the Pharmaceutical Affairs Law, and with clinical application of the products derived from human somatic stem cells, iPS cells, ES cells, and other relevant cells as the goal, the study group concluded that the appropriate relevant guidelines should be tailored to specific cell sources and phenotypes (human autologous versus human allogeneic; somatic stem cells vs. iPS cells vs. ES cells vs. other cells) to facilitate efficient, effective, and rational research and development (R&D). Points to be considered include but are not limited to technical details, the manufacturing process, characterization, quality control, and stability evaluation, and the data necessary to guarantee the safety and efficacy of the products.

With this perspective in mind and with the desire for consistency in scientific principles and concepts, 2 interim reports on draft guidelines on autologous human somatic stem cell-based products and autologous human iPS cell-based products were prepared in 2009 according to Japanese Ministry of Health, Labour and Welfare Notification No. 0208003. Three other interim reports of draft guidelines on allogeneic human somatic stem cell-based products, allogeneic human iPS cell-based products, and human ES cell-based products were also prepared according to Japanese Ministry of Health, Labour and Welfare Notification No. 0912006. These 5 sets of draft guidelines were thoroughly discussed from a variety of viewpoints. They were then widely circulated among interested parties as articles in a relevant scientific journal to allow readers to comment (Hayakawa T., et al.: *Regenerative Medicine* [Journal of the Japanese Society for Regenerative Medicine], 9, 116–180 [2010], in Japanese). Thereafter, these articles were updated and published as 8 articles (Journal of the Japanese Society

for Regenerative Medicine, 10, 86–152 [2011], in Japanese) that served as the basis for the final draft guidelines. After extensive discussions with the study group and after public consultation, the Pharmaceuticals and Food Safety Bureau of the Japanese Ministry of Health, Labour and Welfare issued 5 notifications on September 7, 2012, as described in the previous paper [1].

In the present paper, a continuation of the previous article, we introduce the basic technological requirements for ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of allogeneic human somatic stem cells. The final products derived from the processing of allogeneic human somatic stem cells, which are multipotent and retain the ability to self-replicate, should exhibit cell characteristics different from those of the starting cells as a result of cell processing and may be applied and can function at a site (cell environment) different from where the original cells were localized. These concerns have been added to Notification No. 0912006, which serves as the basis for the present guideline.

Before interpreting and implementing the present guideline, the following points should be considered. The ultimate goal is to provide patients with new therapies that utilize regenerative medicine. The role of the guideline is to present the scientific principles, concepts, ideas, and technical elements that will help to achieve the specified goal in the most efficient and effective manner possible. Because situations, circumstances, and products will vary, the guideline addresses the points of concern in a comprehensive manner. Therefore, it is important to identify the relevant testing parameters and evaluation methods by taking into consideration the characteristics of the cells in question, the specific clinical objective, and the method of application. Those that are applicable should be justified and implemented in an appropriate and flexible manner.

Several points should be kept in mind with respect to the development of medicinal products for regenerative medicine and throughout the implementation of this guideline. The desired products are expected to show a potential as a novel therapeutic method as a result of relevant proof of concept (POC). Relevant data and/or information should establish that there are no critical concerns for product safety that might impede the use of the products in humans for the first time. Thorough observance of the Declaration of Helsinki, including proper informed consent and right of self-determination on the part of the patient, is indispensable.

It should be emphasized again that the primary goal of our endeavor is to offer suitable treatment options as soon as possible to patients with severe diseases that are difficult to treat with conventional modalities. The present guideline should be useful for this purpose. Therefore, it is important to interpret and employ the guideline in a flexible and meaningful way. Stringent observance of the guideline without taking into account the patients and their specific situation (which is like putting the cart before the horse) should be avoided.

It is evident that progress in the application of regenerative medicine is desirable for maintaining and improving human health. The development of innovative and revolutionary medicinal products and therapeutic techniques should benefit our country as well as the international community. Regenerative medicine is a great way to make a peaceful international contribution that will be a legacy for mankind. In this context, the role of the government is to promote clinical research and industrialization; regulations and guidelines are adopted so that we advance towards this common goal in a scientific, rational, efficient, and effective manner. All those involved, like players with a common goal in the same arena, should continue to make efforts to deliver to patients as soon as possible the revolutionary cell-based products and therapeutic techniques.

## Guidelines on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Allogeneic Human Somatic Stem Cells

(Notification No. 0907-3, issued by Pharmaceuticals and Food Safety Bureau, Ministry of Health, Labour and Welfare of Japan, on September 7, 2012.)

### 2. Introduction

1. The present guidelines outline basic technical elements for ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of allogeneic human somatic stem cells. These products are hereafter referred to as allogeneic human somatic stem cell-based products or simply as the “desired cell products.”

There are many types of allogeneic human somatic stem cell-based products and methods of their clinical application. In addition, scientific progress in this field is incessant, while expertise and knowledge are constantly accumulating. Therefore, it is not always appropriate to consider the present guidelines all-inclusive and definitive. Consequently, when testing and evaluating each product, it is necessary to adopt a flexible approach on a case-by-case basis, according to the rationale that reflects the scientific and technological advances at that point in time.

2. The main purpose of evaluating the quality and safety of the desired cell products before conducting investigational clinical trials (e.g., at the time of “clinical trial consultation”) is to determine whether there are any quality and/or safety problems that would obviously hinder initiation of human clinical trials of the allogeneic human somatic stem cell-based products in question, whether certain quality attributes (QA) of the product are understood sufficiently to establish a relationship between the clinical findings and the QA, and whether consistency of the QA can be ensured within a definite range. Simultaneously, it is important to eliminate as much as possible any known risk factors associated with product quality and safety using up-to-date science and technology and to describe the scientific appropriateness of the results of such an action. The remaining presumed risk factors should be weighed against the risks associated with not performing the trials on patients who suffer from diseases that are serious and life-threatening, or that involve marked functional impairment or a marked decrease in quality of life (QOL) resulting from the loss of a certain degree of a physical function or form, or for which existing therapies have limitations and do not result in a cure. Furthermore, it is important to entrust the patient with the right to make a decision after receiving all of the available information. When applying for approval of investigational clinical trials, applicants can submit a provisional nonclinical data package, which is prepared rationally by taking into account product aspects and patient aspects including a balance between the risk of the product vs. the risk facing the patient with/without treatment in question, in order to decide to initiate investigational clinical trials, on the premise that the data package submitted at the time of marketing application/registration to ensure quality and safety will be enriched and developed in line with the guidelines as the clinical trial progresses.

Finally, applicants are encouraged to discuss with the Pharmaceuticals and Medical Devices Agency (PMDA) the type and extent of data that may be needed to initiate an individual clinical trial. Because of differences in product origin, target disease, target patients, application sites, application methods, and processing methods, there may be numerous variations

among individual data packages; these differences cannot be definitively clarified in the present guidelines.

3. The items, test methods, criteria, and any other technical requirements described in the present guidelines are intended to be considered, selected, applied, and evaluated to serve each intended purpose; they do not necessarily require the most stringent level of interpretation and practice. Applicants are encouraged to explain and provide justification for any considerations regarding the background, selection, application, and the content as well as the extent of evaluation that are appropriate for their own purpose and are scientifically valid.

### 3. Chapter I. General principles

#### 3.1. Objective

The present guidelines outline basic technical elements for ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of human allogeneic somatic stem cells (excluding autologous somatic stem cells). These products are hereafter referred to as allogeneic human somatic stem cell-based products or simply as the “desired cell products.”

#### 3.2. Definitions

The definitions of the technical terms used in this guideline are as follows:

1. “Human somatic stem cells”: Cells that are collected from humans or cells that are derived from such cells through cell division and that possess multipotency and maintain the ability to self-renew or a similar ability. In other words, tissue stem cells (e.g., hematopoietic stem cells, neural stem cells, mesenchymal stem cells [including bone marrow stromal stem cells and adipose-tissue-derived stem cells], corneal stem cells, skin stem cells, hair follicle stem cells, intestinal stem cells, hepatic stem cells, and skeletal muscle stem cells) or cell groups that have abundant populations of these cells (e.g., whole-bone-marrow cells that include hematopoietic stem cells), including vascular precursor cells, umbilical cord blood, and bone marrow stromal cells. “Human somatic stem cells” also include cells obtained by culturing these cells in vitro. Human embryonic stem (ES) cells, human induced pluripotent stem (iPS) cells, human induced pluripotent stem-like (iPS-like) cells, human embryonic germ (EG) cells, human multipotent germline stem (mGS) cells, human parthenogenesis stem cells, human nuclear transplant stem cells, human cancer cells, human cancer stem cells, and cells derived from these cells are not included. (Note: The definitions of human ES cells, human iPS cells, and human iPS-like cells are provided in other guidelines, particularly, in “Guidelines on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Human ES Cells” and “Guidelines on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Allogeneic/Autologous Human iPS(-like) Cells,” respectively.)
2. “Processing of cells and tissues”: Any processing of a cell type or tissue, such as propagation and/or differentiation, production of a cell line, activation of a cell by pharmaceutical or chemical treatment, alteration of a biological characteristic, combination with a noncellular component, and manipulation using genetic engineering, with the aim of preparing desired cell products to treat a patient or repair or regenerate tissue.

Isolation of a tissue, homogenization of a tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by  $\gamma$ -irradiation or other methods, freezing, thawing, and other such procedures that are regarded as minimal manipulations are not considered “processing.”

3. “Manufacture”: Actions undertaken before the final product (an allogeneic human somatic stem cell-based product) is released to the market. This includes, in addition to the processing of cells and tissues, minimal manipulations such as separation of a tissue, homogenization of a tissue, separation of cells, isolation of a specific cell, treatment with antibiotics, washing, sterilization by  $\gamma$ -irradiation or other methods, freezing, thawing, and other procedures that do not change the original properties of the cells or tissues.
4. “Phenotype”: A morphological or physiological characteristic that is produced by a certain gene under constant environmental conditions.
5. “HLA typing”: Identification of the type of HLA (human leukocyte antigen), a human primary histocompatibility antigen.
6. “Donor”: A person who donates his/her own cells or tissue, which serve as a raw material for an allogeneic human somatic stem cell-based product.
7. “Transgenic construct”: A construct that contains a vector for introducing a target gene (a specific gene encoding a desired protein or RNA) into a target cell, the target gene itself, and the coding sequences of the elements essential for the expression of the target gene.

## 4. Chapter II. Manufacturing methods

Describe all important and relevant information concerning the manufacturing method, taking into account the items listed below. This information will help to ensure the quality, safety, and efficacy of the final products, and it is important for guaranteeing consistency in quality from the manufacturing perspective. It should be noted that quality, safety, and consistency are ensured by mutual complementary measures throughout the manufacturing process. It is very important that the measures be rational and that they serve the intended purpose. It is acceptable to omit portion of the items listed below, if the quality, safety, and constancy of the final products can be established by suitably chosen quality tests, control of the final or intermediate products, or control of the manufacturing process.

### 4.1. Raw materials and materials used in manufacturing

#### 4.1.1. Human cells and tissues used as raw materials

4.1.1.1. *Source and origin and justification of their selection.* Explain the source and origin of the cells and/or tissues used as raw materials and provide the reasons for selecting these cells and/or tissues.

4.1.1.2. *Characteristics and eligibility of cells and/or tissues used as raw materials*

4.1.1.2.1. *Features of biological structure and function, selection criteria.* Provide and explain the reasons for selecting the cells and/or tissues used as raw materials with reference to characteristics of their biological structure and function, such as morphological characteristics, growth characteristics, biochemical indicators, immunological indicators, specific substances produced, results of HLA typing, and other suitably chosen and appropriate genotype or phenotype indicators (or markers). In particular, demonstrate that the somatic stem cells used as a raw material possess clinically useful stemness. Stemness in this case does not necessarily indicate the potential for multilineage differentiation but refers to the

ability to differentiate into cells that have expected functions in vivo. In addition, although demonstrating differentiation in vitro is desirable, it may suffice to show differentiation in vivo if a rational explanation is provided. For example, when using myocardial stem cells, which are somatic stem cells, as a raw material, it is acceptable to show that myocardial stem cells can differentiate into cardiomyocytes. This should lead to the identification of the critical cell characteristics that will be employed when preparing the somatic stem cells as raw materials.

It is recognized that quantitative technological limits on sample analysis will affect the extent to which such studies can be performed.

4.1.1.2.2. *Donor selection criteria and eligibility.* Indicate that the donor was selected in an appropriate and ethical manner and that the proper procedure was followed. Establish selection criteria and eligibility criteria that take into consideration age, sex, ethnic characteristics, genetic characteristics, the medical history, the health condition, test parameters related to any type of infection that may occur because of cell and/or tissue samples, immunological compatibility, and other characteristics and provide the justification. If donor genomic or gene analysis is undertaken, it shall be performed in accordance with “Ethical Guidelines for Analytical Research on Human Genome/Gene,” issued jointly on February 8, 2013, and revised on November 25, 2014, by the Japanese Ministry of Education, Culture, Sports, Science and Technology; Ministry of Health, Labour and Welfare; and Ministry of Economy, Trade and Industry.

Infection with hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), adult human T-lymphotropic virus (HTLV), or parvovirus B19 shall be ruled out via physician-donor interviews and clinical laboratory tests, such as serological tests and nucleic-acid amplification tests. Infection with cytomegalovirus, Epstein-Barr (EB) virus, or West Nile virus shall also be ruled out, if necessary, via appropriate clinical laboratory tests.

In addition, further assess and determine the eligibility of donors by examining the medical history (mentioned below) of the donor, for example, through physician-donor interviews, and determine whether he/she ever received a blood transfusion or underwent a transplant procedure.

- Bacterial infections, such as syphilis (*Treponema pallidum*), chlamydia, gonorrhea, and tubercle bacillus
- Sepsis or suspected sepsis
- A malignant neoplasm
- Serious metabolic or endocrine disease
- Collagen and blood diseases
- Hepatic diseases
- Confirmed or suspected transmissible spongiform encephalopathy (TSE) or other brain disorders
- A specific genetic disease or a family history of a specific genetic disease

4.1.1.3. *Records related to the donor.* Retain complete records related to the donor so that any information necessary to ensure the safety of cells and tissues that are used as raw materials can be verified. Concrete measures shall be described. For patients and donors of test samples, it is sufficient to prepare and retain only specific information that is related to the intended use of the cells.

#### 4.1.1.4. Collection, storage, and transport of cells and tissues

4.1.1.4.1. *Eligibility of personnel and medical institutions collecting the samples.* Describe the technical requirements for personnel and medical institutions that collect the cells and tissues.



**4.1.1.4.2. Suitability of the sampling site and sampling method.** Describe the rationale for selecting the cell and tissue sampling sites and the sampling method. State why the selected sites are scientifically and ethically appropriate. For cell and tissue sampling methods, indicate the suitability of the equipment and drugs used and the measures adopted to prevent microbial contamination, erroneous sampling (mix-up), and cross-contamination.

**4.1.1.4.3. Informed consent of donors.** Describe the details of the informed consent, including the clinical application, provided by the donor of the cells or tissue.

**4.1.1.4.4. Protection of donor privacy.** Indicate the measures adopted to ensure the protection of the donors' privacy.

**4.1.1.4.5. Tests to ensure donor safety.** If tests such as those to confirm the state of the sampling site need to be performed in order to ensure the safety of the donor at the time of cell or tissue sampling, describe the details of the tests as well as any interventions undertaken when the test results indicate that a problem exists.

**4.1.1.4.6. A storage method and measures to prevent erroneous sampling (mix-up).** If the cells and/or tissues need to be stored for a definite period of time, set the storage conditions and storage period and provide the justification. Describe in detail the measures to be taken to prevent erroneous sampling (mix-up).

**4.1.1.4.7. Transportation methods.** If cells and/or tissues need to be transported, specify the containers used for transport and the transportation procedure (including temperature control) and provide justification for their suitability.

**4.1.1.4.8. Preparation of records and record-keeping procedures.** Prepare written records for items (4.1.1.4.1) through (4.1.1.4.7) above and describe the record-keeping procedures in detail.

#### 4.1.2. Raw materials other than the target cells and tissues, materials used in manufacturing

Describe raw materials other than the target cells and tissues as well as other materials used in the manufacturing process; indicate their appropriateness for the intended use; and, if necessary, establish their specifications (a set of acceptance criteria and analytical procedures). Proper quality control of these materials should be implemented.

When so-called Biological Products, or Specific Biological Products (refer to Articles 2.9 and 2.10 of the Pharmaceutical Affairs Law) are used as raw materials, use the minimum amount required and strictly conform to the relevant laws and regulations, such as “Standards for Biological Raw Materials” (Notification No. 210, Japanese Ministry of Health, Labour and Welfare, 2003; a partially revised version was issued on September 26, 2014). It is particularly important to adequately evaluate information related to the inactivation and elimination of viruses and to specify measures for encouraging retrospective and other studies.

##### 4.1.2.1. When culturing cells

- (i) Indicate the appropriateness of all media components including such as additives (e.g., serum, growth factors, and antibiotics), and reagents, used in the treatment of cells and set specifications if necessary. Give consideration to the route of clinical application and other parameters of the final product when setting specifications concerning the appropriateness of each component.
- (ii) Take into consideration the following points with respect to media components:
  - (a) The ingredients and water used in media should be of high quality and high biological purity, and their quality should be controlled using standards equivalent to those

used with pharmaceuticals and pharmaceutical ingredients.

- (b) Provide information on all ingredients used in the media as well as the rationale for their selection, and, if necessary, the quality control and other procedures. However, widely known and commercially available media products such as DMEM, MCDB, HAM, and RPMI are regarded as a single raw-material set.
  - (c) Conduct sterility tests and performance tests on media that contain all components in order to determine whether they are suitable as target media. Set specifications for any other relevant parameters expected to be controlled in the process and perform proper quality control.
- (iii) Heterologous serum or components derived from heterologous or homologous serum shall not be used unless they are essential for processes such as cell activation or cell growth. In particular, for products that may be used repeatedly, investigate, to the extent possible, ways to avoid using these serum components. If the use of serum or other such materials is unavoidable, consider the following points and investigate ways to prevent the contamination and the transmission of bacteria, fungi, viruses, and prions from serum and other related materials as well as treatment methods for their elimination, to the extent possible, from the final product.
    - (a) Clarify the origin of the serum or other components.
    - (b) Make strenuous efforts to minimize the risk of prion infection, e.g., by strictly avoiding the use of serum from areas or regions with known outbreaks of bovine spongiform encephalopathy (BSE).
    - (c) Use these batches of serum only after confirming that they are not contaminated with viruses or other pathogens by conducting appropriate tests to prove the absence of specific viruses and mycoplasma that originate in animal species.
    - (d) Perform appropriate procedures to inactivate and eliminate bacteria, fungi, and viruses to an extent that does not affect activation and growth of the cells. For example, to avoid the risks associated with latent viral contamination, perform combinations of heat treatment, filtration,  $\gamma$ -irradiation, and/or ultraviolet light treatment, if needed.
    - (e) Preserve and store a portion of the serum used in order to monitor cultured cells for viral infections, to monitor onset of viral diseases among the patients, and measure antigen production in response to a component of the heterologous serum used.
  - (iv) When using feeder cells, conduct quality evaluation while referring to “Derivation and Characterization of Cell Substrates Used for the Production of Biotechnological/Biological Products” (Pharmaceutical Notification No. 873, issued July 14, 2000, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare), “Guidelines on Public Health Infection Issues Accompanying Xenotransplantations” (Notification No. 0709001, issued July 9, 2002, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labour and Welfare), and “Guidelines on Epithelial Regenerative Therapy Using 3T3J2 Strain or 3T3NIH Strain Cells as Feeder Cells” based on “Guidelines on Public Health Infection Issues Accompanying Xenotransplantations” (Notification

No. 0702001, issued July 2, 2004, Research and Development Division, Health Policy Bureau, Japanese Ministry of Health, Labour and Welfare) in order to prevent contamination of feeder cells and the transmission of bacteria, fungi, viruses, and prions. Indicate methods for the inactivation of cell division potential and conditions such as cell density when using the feeder cells. However, if, for example, the feeder cells or equivalent cells are being used in the manufacture of a cell or tissue product that has already been used clinically and whose characteristics and microbiological safety have already been assessed and confirmed, it is possible to omit the virus tests or parts of other tests by demonstrating the appropriateness of these cells.

- (v) Avoid the use of antibiotics as much as possible. However, if the use of antibiotics at the initial stages of processing is deemed indispensable, attempt to decrease their use at subsequent steps as much as possible, and clearly indicate the appropriateness of their use from perspectives such as the scientific rationale, estimated residual amounts in the final product, and the effects on the patient. If it has been determined that an antibiotic can be adequately eliminated, its use does not need to be restricted. On the other hand, if a patient has a history of allergy to the antibiotic used, in principle, this therapeutic method should not be used. If there is no way to avoid the use of antibiotics, administer them very carefully and obtain informed consent from the patient.
- (vi) If growth factors are used, show the appropriate quality control methods using relevant parameters, such as purity and potency, for which established acceptance criteria and assay methods are employed, in order to guarantee the reproducibility of the cell culture characteristics.
- (vii) For media components and other components that are used in processing and that may contaminate the final product, choose components that do not have any harmful biological effects.

#### 4.1.2.2. When combining cells with noncellular components

4.1.2.2.1. *Quality and safety of noncellular raw materials.* If the final product consists of cells and noncellular components such as a matrix, medical materials, scaffolds, support membranes, fibers, and beads, clearly describe in detail the quality and safety of the noncellular components.

Provide any relevant information concerning the noncellular raw materials, taking into consideration their type and characteristics, the form and function in the final product, and evaluation of the quality, safety, and efficacy from the standpoint of the presumed clinical indication. If using materials that are absorbed by the body, perform the necessary tests on the degradation products to address safety concerns.

With respect to the tests that should be carried out, refer to “Basic Views on Biological Tests Necessary for Regulatory Approval for Manufactured or Imported Medical Devices” (Notification No. 0213001, issued February 13, 2003, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare), describe the test results, and provide justification for the use of such raw materials. Use of information from scientific literature is encouraged.

4.1.2.2.2. *Interactions with target cells.* Demonstrate the validity of the tests used and provide justification for the results obtained

for the following 3 items with respect to the interactions between noncellular components and cells in the final product and in any intermediate products.

- (a) The noncellular components do not have any deleterious effects on the function, growth capacity, activity, or stability of the cells in the final product required for the presumed clinical indication or the cells in any intermediate products.
- (b) Evaluate to the extent possible any potential interactions between the cells and noncellular components, taking into consideration, for example, the mutation, transformation, and/or dedifferentiation of the cells in the final product or cells in intermediate products.
- (c) Show that there is no loss of the expected properties of the noncellular components for the presumed clinical indication as a result of any interactions between the noncellular components and the cells in the final and intermediate products.

4.1.2.2.3. *When using noncellular components to isolate the desired cell products from the application site.* When using noncellular components with the objective of segregating the cells from the application site, confirm their usefulness and safety by referring to items (a) through (e) below.

- (a) When immunological segregation is the objective, describe its level.
- (b) Membrane permeability kinetics and the pharmacological effects of target physiologically active substances derived from the cells in the final product.
- (c) Diffusion of nutritional components and excretory products.
- (d) Effects of noncellular components on the area near the application site.
- (e) When a pharmacological effect of a target physiologically active substance derived from a desired cell product is anticipated and the objective is segregation of the application site and the desired cell product or undifferentiated cells, confirm that the cells do not leak out, which might result, for instance, from the degradation of noncellular components.

4.1.2.3. *When cells are subjected to genetic modifications.* When genes are introduced into cells, provide the following details:

- (i) For the target gene (the specific gene encoding a desired protein or RNA), information related to its structure and origin, the method by which it was obtained, cloning methods, and methods of cell bank preparation, control, renewal, and other relevant information.
- (ii) Nature of the transgene.
- (iii) Structure, biological activity, and properties of the desired protein or RNA derived from the target gene.
- (iv) All raw materials, their properties, and procedures (transgenic method, origin, and properties of the vector, and method for obtaining the vector used for introduction of the transgene) needed to produce the transgenic construct.
- (v) Structure and characteristics of the transgenic construct.
- (vi) Control and preparation methods for cell and virus banks that are used to prepare vectors and transgenic constructs.

For manufacturing methods for transgenic cells, refer to Chapter 2 and other sections of “Guidelines for Ensuring the Quality and Safety of Gene Therapy Pharmaceuticals,” which is an appendix in “Concerning Guidelines for Ensuring the Quality and Safety of Gene Therapy Pharmaceuticals” (hereafter referred to as “Gene Therapy

Pharmaceutical Guidelines”), published as Notification No. 1062 by Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare on November 15, 1995. In addition, state the appropriateness of the establishment of a cell line in accordance with the appendix of the same notification.

On the basis of the law (Law No. 97, 2003) implemented to ensure biodiversity by regulating the use (and other aspects) of genetic recombination-derived organisms, a separate application procedure for evaluation will be required when living organisms, including certain cells, “viruses,” and “viroids,” are genetically modified. The following cells are not regarded as living organisms: “human cells” or “cells that have the ability to differentiate or differentiated cells that are not viable when alone under natural conditions.”

Regardless of the guidelines mentioned above, if a gene introduced into cells is used as a reagent in the manufacturing process and does not contribute either chemically or functionally to the final product, it is acceptable to describe, on the basis of current knowledge, how the quality and safety of the gene conform to the intended use.

## 4.2. Manufacturing process

When manufacturing allogeneic human somatic stem cell-based products, describe in detail the manufacturing method and verify, to the extent possible, the appropriateness of the method, using the items listed below, in order to maintain consistency in the quality of the product.

### 4.2.1. Lot control

Indicate whether a lot control procedure is applied for final products and intermediate products. If any lot control procedure is adopted, establish standardized procedures for the makeup and control of the lot, which may include the lot size, labeling/numbering, testing method and acceptance criteria.

### 4.2.2. Manufacturing method

Provide an outline of the manufacturing method, from the receipt of the cells and tissues to the isolation of somatic stem cells and the establishment of the final product. Describe the technical details of the process and necessary process control and product quality control.

**4.2.2.1. Tests upon receipt.** Establish a battery of tests as well as acceptance criteria for assessing the suitability of the cells and tissues that will serve as the raw materials, taking into account the nature of the cells and their intended use. These may include, for example, visual tests, microscopic examination, recovery factors of target cells, cell viability assays, characterization of cells and tissues, and microbiological tests. At the stage of initiating clinical trials, provide the actual measured values obtained up until that point with test samples, and propose a provisional set of acceptance criteria based on these values.

**4.2.2.2. Inactivation and elimination of bacteria, fungi, viruses, and other microorganisms.** In cells and tissues that serve as raw materials, inactivate bacteria, fungi, viruses, and other microorganisms, if necessary and whenever possible, to the extent possible, without altering the cellular characteristics (e.g., cell viability, phenotype, genetic traits, and specific functions) and quality of the cells and tissues serving as raw materials. State the suitability of the measures, procedures, and evaluation methods used if any.

**4.2.2.3. Tissue homogenization, cell separation, isolation of specific cells, and other techniques.** Describe the methods for

homogenization of tissues, separation of cells, and isolation of somatic stem cells as well as methods for washing of the cells and tissues (and other methods), that are performed at the early stages of manufacturing of somatic stem cell-based products from collected cells and tissues. When isolating somatic stem cells, establish methods of cell identification.

**4.2.2.4. Preparation of cells that are a principal component and active ingredient in the final product.** Describe the methods used to collect human cells and tissues, to isolate somatic stem cells, and to obtain the cells that serve as the active ingredient in the final product. The items to be described include induction of differentiation, isolation, and culture of the desired cells, and preparation of the media, culture conditions, the culture period, and the yield of the cells at each step. Describe the appropriateness of each method.

**4.2.2.5. Establishment and use of cell lines.** Establish cell lines after having determined to the extent possible the genetic background of the donor. Describe the method of establishment of a cell line and its appropriateness to the extent possible.

To ensure that the quality of the established cell line remains stable and consistent, identify the critical quality attributes of the cells (e.g., cell population purity, morphological features, phenotypic markers, karyotype, cell growth properties, and differentiation potency) and set acceptance criteria for them. In addition, demonstrate the number of passages or cell division within which the cells remain stable in terms of the criteria specified.

Discuss the possibility of tumorigenicity and malignant transformation, using an appropriate animal model, where necessary and appropriate.

**4.2.2.6. Establishment of cell banks.** When a cell bank is established at any stage during the manufacture of allogeneic human somatic stem cell-based products, describe the rationale for preparing the cell banks; the methods used to prepare the cell banks; the characteristics of the cell banks, and the storage, maintenance, control, and renewal methods as well as any other processes and tests performed. Provide justification for each. Refer to “Derivation and Characterisation of Cell Substrates Used for the Production of Biotechnological/Biological Products” (Pharmaceutical Notification No. 873, issued July 14, 2000, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Japanese Ministry of Health, Labour and Welfare) and other documents.

**4.2.2.7. Measures to prevent erroneous sampling (mix-up) and cross-contamination during the manufacturing process.** It is extremely important to prevent erroneous sampling and cross-contamination during the manufacturing process when manufacturing the allogeneic human somatic stem cell-based products. Therefore, describe preventive measures for the process.

### 4.2.3. Characterization of cells that are a principal component and active ingredient in a final product

For such cells, analyze the attributes, such as cell population purity (to control contamination by nontarget cells), cell viability, morphological characteristics, cell growth characteristics, biochemical markers, immunological markers, distinctive substances produced by the cells, karyotype, and other appropriate genotypic and phenotypic markers. In addition, characterize their biological functions if necessary. Furthermore, to evaluate the appropriateness of the culture duration and the stability of the cells, use appropriate markers of cell characteristics to demonstrate that there have been no unintended changes in cells cultured longer than the proposed culture period. It is acceptable to perform these studies preliminarily, using test samples obtained from



donors in place of the real products that will be prepared for a clinical trial. On the basis of these test results, identify the critical cell characteristics that should be used when applying the product to a patient. Although comprehensive cell characterization is always desirable, it is not always possible to characterize the cells fully because there are quantitative limits on sample analysis. Thus, it is acceptable to perform a limited study to the extent possible. When cell processing, such as growth within the body, is anticipated after a clinical application, clearly demonstrate the functions expected by describing the specified criteria with respect to the passage number or number of cell divisions.

#### 4.2.4. *The form and packaging of the final products*

The form and packaging of the final product shall ensure the quality of the final product.

#### 4.2.5. *Storage and transport of the final product*

If an intermediate or final products need to be stored and transported, the storage procedure and duration, the containers used for transport, and the transportation procedure (including temperature control) shall be stated and their suitability clearly indicated (refer to Chapter III).

#### 4.2.6. *Consistency of the manufacturing procedure*

To assess the consistency of the manufacturing process using each product (or each lot, if any) obtained from different production runs, determine whether they differ significantly with respect to the number of cells, cell viability, and cell characteristics (such as relevant markers of a phenotype and genotype, functional characteristics, and the percentage of the desired cells) and from the point of view of the clinical application methods and the intended clinical use of the product. It is acceptable to use test samples obtained from donors in place of the real product that will be prepared for a clinical trial. Evaluation of intermediate products may provide insight into the suitability of cells and tissues for use as raw materials and the validity of the manufacturing process up until the intermediate-product stage and can provide an appropriate guidepost en route to the final product. Therefore, it may be reasonable to adopt such an approach, where necessary and appropriate.

When the manufacturing process involves long cryopreservation periods or cell cultivation periods, perform sterilization tests at constant intervals to confirm that sterility has been maintained.

#### 4.2.7. *Changes in the manufacturing process*

If the manufacturing process is altered at some point during development and the test results that are obtained using products manufactured before the change in the manufacturing method are to be used in the application for clinical trial or regulatory approval, demonstrate that the products manufactured before and after the change to the manufacturing process are comparable.

### 4.3. *Quality control of the final product*

#### 4.3.1. *Introduction*

The overall quality control strategy for products manufactured using human somatic stem cells include specifications (a set of acceptance criteria and analytical procedures) for the final products, quality control of raw materials for each therapeutic application to patients, verification of the suitability of the manufacturing process, maintenance of consistency, and proper quality control of any intermediate products.

Specifications will differ among final products, depending on the type and properties of the desired cells and tissues, manufacturing methods, intended clinical use, methods of clinical

application, stability, and test methods. These differences shall be taken into consideration when setting the acceptance criteria and test procedures. In addition, specifications shall be set and justified from the standpoint of achieving the purpose of quality control as a whole, by taking into consideration the mutually complementary relationships among 1) verification of the suitability of the manufacturing process, 2) the method for maintaining consistency, and 3) quality control of the raw materials and intermediate products. The purpose of the assessment at the initiation of clinical trials is to confirm that the product is unlikely to pose significant quality/safety problems for use in investigational clinical trials. Therefore, it is possible to set provisional specifications with allowance for some variation on the basis of the measured values obtained for a few test specimens, as long as one can explain about the relationship between the results of clinical tests and the quality attributes after the clinical trial. However, testing for sterility and the absence of mycoplasma is essential. It should be noted that the quality control strategy, including specifications, should be enriched and developed as the clinical trial progresses.

#### 4.3.2. *Quality control of the final product*

Refer to the general quality control parameters and tests shown below, set necessary and appropriate specifications for the final product, and provide the rationale for the specifications set.

Set appropriate acceptance criteria and test procedures for individual products that do not make up a lot and for products that do make up a lot because normally each lot is the unit subject to quality control.

**4.3.2.1. *Cell number and cell viability.*** For cells that are an active ingredient in the final product, determine the cell number and viability in the final product or, if needed, in an appropriate intermediate product. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

**4.3.2.2. *Tests of identity.*** Confirm that the cells are the intended target cells by assessing important cell characteristic(s), such as morphological characteristics, biochemical markers, immunological markers, characteristic products, and other appropriate genotypic or phenotypic features.

**4.3.2.3. *Tests of purity.*** To test the purity of the cell population in a final product, if necessary, set the test parameters, test methods, and acceptance criteria for evaluating and controlling nontarget cells, such as undifferentiated cells, cells that exhibit abnormal growth, transformed cells, and contaminating cells, taking into consideration such parameters as the origin of the target cells and tissues, the culture conditions, other parameters of the manufacturing process, quality control of intermediate products. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

**4.3.2.4. *Tests for cell-derived undesirable physiologically active substances.*** Specify appropriate tests for determining the permissible dose limit of any potential undesirable physiologically active substances that are derived from the target cells if the presence of such substances in the product is presumed to clearly affect the safety of the patient. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

**4.3.2.5. *Tests for process-related impurities.*** For substances that may be present in the final product as, for example, contaminants,

residues, newly generated products, or degradation products; that potentially originate from raw materials, noncellular components, media ingredients (including feeder cells), chemical reagents, or any other process-related materials; and that may have deleterious effects on quality and safety (for example, albumin derived from fetal calf serum and antibiotics), it is necessary to 1) prove that the substance is not present in the final product by taking into consideration the results of process evaluations related to the elimination of the substance or the results of in-process control of the substance or 2) establish appropriate tests to control permissible levels of the substance in the final product. When selecting substances to be tested and setting their acceptance criteria, their suitability should be explained and justified. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

**4.3.2.6. Sterility tests and tests for the absence of mycoplasma.** Sterility should be ensured throughout the entire manufacturing process by evaluating test samples. The sterility (negative results of tests for common bacteria and fungi) of the final product should be demonstrated before its use in a patient. Appropriate tests confirming the absence of mycoplasma should also be performed. A validated nucleic-acid amplification test can be used. If the results of sterility and other tests on the final product can be obtained only after the product is administered to the patient, methods for dealing with the lack of sterility detected after administration should be established beforehand. In such cases, demonstrate by testing that the intermediate products are sterile and that sterility has been strictly maintained in all processes leading to the final product. If a product from the same facility and the same process has already been used in patients, its sterility must be confirmed by testing all patients. If complete closure (hermetic seal) of a product that is a part of a lot has been ensured, tests on representative samples are sufficient. When each different application needs to be tested and if the test results can be obtained only after administration to the patient, the decision to administer the product will be based on the most recent data. However, even in such cases, the final product shall be tested.

It is desirable that every effort be made to avoid the use of antibiotics in cell culture systems. However, if antibiotics are used, adopt measures to ensure that they do not influence the sterility tests.

**4.3.2.7. Endotoxin test.** Perform the endotoxin test, taking into consideration the impact of the contaminant in the samples. The acceptance criteria do not necessarily depend on the actual measured values. Set acceptance criteria by taking into consideration the safety ranges in the Japanese Pharmacopoeia and/or any other relevant compendia that are based on a single dose of the final product. Endotoxin testing can be established as an in-process control test. However, in such cases, specify the criteria, including the validation results, and provide the justification.

**4.3.2.8. Virus tests.** Use a titer test to detect viruses in the intermediate and final products, when using cells that are not banked; that are from donors not tested during the infection window; and in which HBV, HCV, HIV, or HTLV can propagate. If materials of a biological origin are used in the manufacturing process, it may be necessary to test the final product for viruses originating from those components. However, whenever possible, it is preferable to determine that there is no contamination by testing at the original component stage or by process evaluation.

**4.3.2.9. Specific biological tests.** In some instances, it will be necessary to consider specific (quantitative or qualitative) biological testing that takes into account the cell type, intended clinical use, or distinctive characteristics of the cells. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

**4.3.2.10. Potency assay.** If a specific physiologically active substance that is secreted from cells or tissues contributes to the clinical efficacy or effect of an allogeneic human somatic stem cell-based product, establish test parameters and/or acceptance criteria for the substance in order to demonstrate the intended effect. Set acceptance criteria for potency or quantitation of a gene expression product secreted from the cells when a transgene was introduced. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

**4.3.2.11. Mechanical compatibility tests.** For products that require a certain degree of dynamic strength, set acceptance criteria for mechanical compatibility and durability that take into account the site of application. At the beginning of the clinical trial, it is acceptable to set provisional acceptance criteria that are based on measured values obtained for a small number of test samples.

## 5. Chapter III. Stability of allogeneic human somatic stem cell-based products

Taking into full consideration the storage and distribution periods and the storage form, test the cell viability, potency, and other characteristics of allogeneic human somatic stem cell-based products and/or critical intermediate products to establish storage methods and an expiration date. Provide justification for their suitability. In particular, when product storage and use involves freezing and thawing, confirm that the freezing and thawing processes do not affect the stability or acceptance criteria of the product. Where necessary and possible, conduct stability studies on products whose manufacturing period or storage period exceeds normal periods in order to confirm, to the extent possible, the limits of stability. This does not apply if a product will be used immediately after its production.

If an allogeneic human somatic stem cell-based product will be transported, the relevant transportation vessels and transportation procedures (such as thermal management) shall be set and their appropriateness justified.

## 6. Chapter IV. Preclinical safety testing of allogeneic human somatic stem cell-based products

To the extent that they are scientifically reasonable and technically possible, relevant animal tests and/or in vitro tests may be performed in order to address safety concerns associated with an allogeneic human somatic stem cell-based product. For noncellular constituents and process-related impurities, safety concerns should be addressed as much as possible by physicochemical analyses not by animal testing.

Testing of human specimens is very valuable, whereas testing the products of human origin in experimental animals does not always yield meaningful results. Thus, there may be a scientific rationale for preparing products of animal origin and testing them in appropriate experimental animals if such a test system is expected to generate useful results. In such a case, consider using an animal model that is suitable for the target disease. (For example, monkeys may be suitable for studies of neurological diseases, and

pigs and/or dogs may be suitable for studies of cardiovascular diseases.) However, because cells with characteristics identical to those of cells that constitute an allogeneic human somatic stem cell-based product cannot necessarily be obtained from nonhuman animal species, even if the preparation procedures are the same, and because a product of animal cell origin that is manufactured using identical processes will not necessarily be comparable to a human cell product, applicants should conduct a feasibility study before adopting, conducting, and evaluating such tests. When performing animal experiments using somatic stem cell-based products obtained from nonhuman animal species, explain why extrapolation to humans is appropriate. Depending on the case, consider test systems that employ cells, and clearly explain the suitability of the test system.

Presented below are items and points to consider and to refer to when confirming the preclinical safety of a product. These examples are provided for illustration purposes; they are not intended to prescribe tests for which there is no rational basis. Taking into consideration the allogeneic origin of the cells, the characteristics of the product, the intended clinical use, and other parameters, conduct necessary and appropriate tests, and evaluate and discuss the results in a comprehensive manner.

1. For cells expanded beyond the limit set for routine cultivation (defined by duration of culture, the population doubling level, or the passage number), demonstrate that alterations other than those intended have not occurred.
2. It may be necessary to quantify special physiologically active substances produced by the cells and tissues and to discuss their effects when they are administered to patients. In some cases, significant amounts of active substances including cytokines and growth factors would be produced by the cells, potentially resulting in undesirable effects on the patients.
3. From the standpoint of product safety, examine and discuss the potential effects of the product on a patient's healthy cells and tissues and the consequences.
4. Depending on the type of product, investigate and discuss the possibility of ectopic tissue formation by the cells in the product and the potential safety consequences thereof when the product is administered to the patient.
5. Investigate and discuss the possibility of undesirable immunological reactions caused by the product and/or the expression product of a transgene and the consequences thereof.
6. Discuss in a comprehensive manner the possibility of tumor formation, including benign tumors and/or malignant transformation, taking into consideration the type and characteristics of the product, number of cells, route of administration, mode of application (e.g., cell sheet or cell suspension), cell engraftment site, target diseases, and suitability of the test systems, among other parameters. If necessary, conduct studies on a suitable animal model. If there is a possibility of tumorigenicity or malignant transformation, provide justification for the use of the product in question, taking into consideration the anticipated efficacy. (Note: For tumorigenicity studies, it is very important to accurately assess tumorigenicity of the final product that will be used in patients. However, tumorigenicity may have to be evaluated using cells from the intermediate product if, for various reasons such as an insufficient cell number, the cells in the final product cannot be used. Furthermore, when conducting tumorigenicity tests in animal models, variables such as cell dispersion, cell adhesion to scaffolding, cell density, and the administration site may not be the same as those of the final product. The species, strain, and immunological state of the animal may also affect its sensitivity. The tumorigenicity of the final product should be evaluated in a comprehensive manner

taking these factors into account.) The risks to the patient arising from tumorigenicity of the final product should be evaluated by weighing the risks of treatment against the benefits of treating the disease.

7. If an exogenous gene is introduced into certain cells during the manufacturing process, conduct tests in accordance with "Gene Therapy Pharmaceutical Guidelines". In particular, if viral vectors are used, perform quantitative tests to determine whether any replication-competent viruses are present, and provide justification for the test employed. Describe the safety of the transgene and its products based on their characteristics. For cells, discuss the possibility of changes in cell growth and of tumor formation, including benign tumors and malignant transformation. When using a vector that can get inserted into a chromosome, consider the necessity of evaluating abnormal proliferative characteristics and/or tumorigenicity and implementing long-term follow-up.
8. Consider conducting rationally designed general toxicological tests if the product, including an animal-derived product, is easy to obtain and if doing so will produce useful information regarding its clinical application. When conducting general toxicological tests, refer to "Guidelines for Toxicology Studies on Pharmaceuticals," which is an appendix in the document entitled "Guidelines on Toxicology Studies Required for Regulatory Approval for the Manufacture or Import of Pharmaceuticals" (Drug Evaluation Notification 1:24, issued September 11, 1988, New Drug Division/Evaluation and Licensing Division, Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare).

## 7. Chapter V. Studies supporting the potency or efficacy of allogeneic human somatic stem cell-based products

1. A well-designed study on experimental animals and/or cells should be performed to demonstrate, to a scientifically reasonable and technically possible extent, the functional expression, the sustainability of effects, and/or anticipated clinical efficacy (proof of concept) of an allogeneic human somatic stem cell-based product.
2. For transgenic cells, demonstrate the expression efficiency, sustainability of expression, and biological activity of the desired products derived from the transgene. Discuss the rationale of the transgene expression products as active ingredients for anticipated clinical efficacy (proof of concept) of the allogeneic human somatic stem cell-based product in question.
3. Where appropriate products derived from the processing of animal somatic stem cells and/or disease model animals are available, use them to study the potential therapeutic efficacy of the product.
4. At the beginning of the clinical trial, detailed experimental studies will not necessarily be required if scientific literature and/or other information supports the prediction that the potency or efficacy of the product in question will be markedly superior to that of a different therapeutic method.

## 8. Chapter VI. Pharmacokinetics of allogeneic human somatic stem cell-based products

1. Pharmacokinetic studies of the internal behavior of transgene expression products or cells/tissues that constitute the final products (these studies may include assessment of the absorption and distribution in experimental animals), should be

performed to an extent that is technically possible and scientifically valid. Thereby, these experiments are expected to estimate the survival of cells/tissues administered to patients and the duration of their effect and to determine if the intended efficacy is successfully achieved. (Note: Testing methods may include histological studies, human Alu sequences amplification by polymerase chain reaction (Alu-PCR), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), and bioimaging.)

2. For allogeneic human somatic stem cell-based products, clarify, in animal studies, the rationale for the administration method. In particular, extrapolate from animal experiments the systemic distribution of the cells after systemic administration and discuss the distribution from the point of view of clinical usefulness. (Note: Although it is unclear exactly where the cells adhere with each administration route, local administration is presumed to be preferable to systemic administration. However, if the benefits to patients can be explained, it is acceptable to use systemic administration. In any case, an administration method that minimizes the distribution of a somatic stem cell-based product to organs other than the target organ would be a rational choice. Even if the cells do localize to a site other than the intended transplantation site, this administration method may be used if no effects on patients result. Arrhythmia caused by osteogenesis of mesenchymal stem cells that ectopically locate to the heart is an example of an adverse effect that can result from ectopic differentiation.)
3. When the cells or tissues are directly applied or targeted to a specific site (e.g., tissue) where they are expected to act, clarify the localization, and discuss the effect of the localization on the efficacy and safety of the product.

## 9. Chapter VII. Preliminary analysis of clinical trials

The main purpose of the present guideline is to outline points to consider for evaluating the quality and safety of allogeneic human somatic stem cell-based products, at the time of application for marketing authorization or at the beginning of an investigational clinical trial. In the latter case, it is necessary to determine, while taking into consideration the clinical usefulness, whether there are any quality and/or safety problems that might impede the initiation of human clinical trials. Thus, quality and nonclinical safety evaluation for a decision to initiate the investigational clinical trials of the product in question should be conducted in reference to the points outlined below. Any presumed risk factors associated with product quality and safety should be eliminated, as much as possible, using up-to-date scientific and technological methods, and the scientific appropriateness should be clearly described. Any remaining risks should be weighed against the risks associated with not performing the trials on patients that suffer from diseases

that are serious and life-threatening, that involve marked functional impairment or a marked decrease in quality of life (QOL) resulting from the loss of physical function or form, or for which existing therapies have limitations and do not result in a cure. Furthermore, it is necessary to entrust the patient with the right to make a decision after receiving all of the information available, including all information on identified/presumed risks and anticipated benefits.

1. Target disease.
2. Target subjects and patients who should be excluded as participants.
3. Details of the therapy to be performed in the subjects, including the application of allogeneic human somatic stem cell-based products and drugs used concomitantly. (Note: If it is anticipated that drugs will be co-administered in order to maintain, enhance, and/or induce the function of administered or transplanted cells, verify the intended activity of the drugs either in vitro or in vivo.)
4. The rationale for conducting the clinical trials in light of existing therapeutic methods.
5. Plan on explaining the clinical trial to the patients, including the currently known risks and benefits of the product.

Clinical trials should have an appropriate study design and clearly specified endpoints. They should be designed in light of the desired cells/tissues, target disease, and method of application.

## Disclosures

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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